Abstract of the Disclosure

A heterogeneous binding assay for an analyte in a fluid sample is developed, which uses a green fluorescent protein (GFP) label. A ligand-GFP conjugate has a specific binding affinity for an anti-ligand immobilized on a support. The anti-ligand also has a specific binding affinity for the analyte. Competition between the analyte and ligand-GFP conjugate for binding sites on the anti-ligand permits an assay for an unknown amount of the analyte. Preferred specific binding pairs for use in the assay are biotin:avidin, and a selected antibody and its antigen. A preferred assay employing an antibody and its antigen is illustrated for a fusion protein containing GFP and an antigenic determinant. Picomolar amounts of analyte can be detected.

The mutant of GFP that contains a six-histidine tail to facilitate purification on an immobilized metal affinity column is chemically modified to incorporate biotin moieties. The resulting conjugates retain the fluorescence characteristics of the unmodified protein and are used along with avidin-coated magnetic beads in the development of the assay.